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(21) International Application Number: PCT/US92/06681 (22) International Filing Date: 10 August 1992 (10.08.92) (30) Priority data: 744,619 12 August 1991 (12.08.91) US (71) Applicant: RESEARCH CORPORATION TECHNOLOGIES, INC. [US/US]; 6840 East Broadway Boulevard, Tucson, AZ 85710 (US). (72) Inventors: HOUGHTON, Peter, J. ; 1718 Overton Park, Memphis, TN 38112 (US). HORTON, Julie, K. ; 11 Observation Court, #301, Germantown, MD 20876 (US). THIMMAIAH, Kuntebommanahalli, N. ; 1138 Lalithadri Road, II Cross, Kuyempunagar, Mysore-570023 (IN).		(74) Agent: SCOTT, Anthony, C.; Scully, Scott, Murphy & Presser, 400 Garden City Plaza, Garden City, NY 11530 (US). (81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE). Published <i>With international search report.</i>
(54) Title: N-SUBSTITUTED PHENOXAZINES FOR TREATING MULTIDRUG RESISTANT CANCER CELLS (57) Abstract Phenoxazines, unsubstituted or N-substituted as defined herein, can potentiate the antitumor effectiveness of chemotherapeutic agents, particularly in multiple drug resistant (MDR) cells.		

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N-SUBSTITUTED PHENOXAZINES FOR
TREATING MULTIDRUG RESISTANT CANCER CELLS

The present invention is directed to chemotherapy of cancer.

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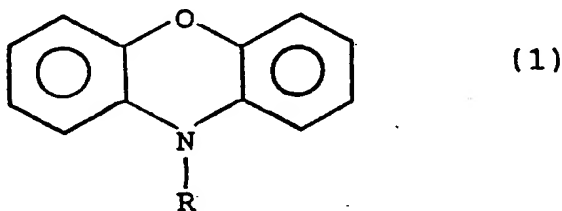
A major reason for failure of treatment of cancer patients is resistance to conventional chemotherapeutic agents. One type of drug resistance, called multi-drug resistance (MDR) is characterized by cross-resistance to functionally and structurally unrelated chemotherapy drugs, such as doxorubicin, vincristine (VCR), vinblastine (VLB), colchicine, and actinomycin D. A number of drugs appear to be active in modifying MDR in model systems, including the calcium channel blocker, verapamil (VRP), the calmodulin inhibitor, trifluoperazine, the anti-arrhythmic drug, quinidine, reserpine, cyclosporin A, Vinca alkaloid analogs, dihydropyridines, and pyridine analogs. Thus, it can be seen that agents that reverse MDR apparently do not seem to have common features. Although several of these MDR-reversing agents have been or are now being tested clinically in cancer patients, they have largely failed to enhance sensitivity to the chemotherapeutic agent. Instead, serious toxicities develop at or below plasma drug levels required for MDR reversal in vitro.

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A tricyclic compound, phenoxazine, has been found to potentiate the uptake of VCR and VLB in MDR GC₃/Cl and KBCh^R-8-5 cells to a greater extent than verapamil. While this discovery has utility and holds promise, it would be desirable to identify derivatives of phenoxazine which would modulate MDR and which show even higher stability and lower toxicity.

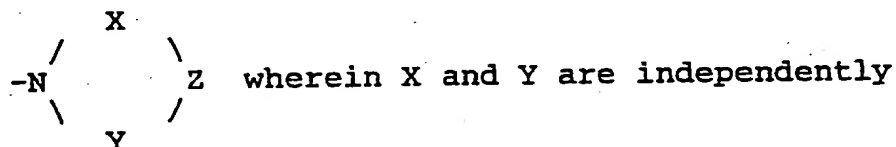
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1 In one aspect, the present invention comprises
compounds of formula (1):



5 and pharmacologically acceptable salts thereof,
wherein R is $-[C(O)]_a-(CH_2)_b-A$; wherein a is 0 or 1 and
10 b is an integer from 0 to 6, provided that a and b are
not both zero;

A is selected from the group consisting of
-NR₁R₂ wherein R₁ and R₂ are independently
alkyl having 1 to 4 carbon atoms, and either or both of
15 R₁ and R₂ are optionally substituted with -OH;

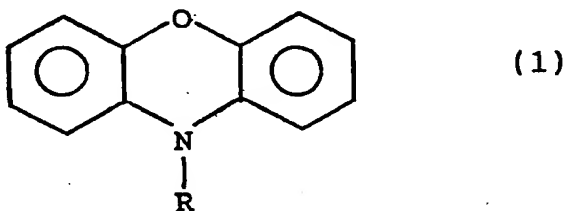


20 alkylene having 1 to 4 carbon atoms, and Z is -O-,
-N(R₃)- or -CH(R₄)-, wherein R₃ is hydrogen or alkyl
having 1 to 4 carbon atoms optionally substituted with a
hydroxyl group, and wherein R₄ is hydrogen or alkyl
having 1 to 4 carbon atoms optionally substituted with a
25 hydroxyl groups;

halide; and trihalomethyl.

The present invention also relates to a method
of potentiating the cytotoxicity of an agent cytotoxic
to a tumor cell, comprising administering to said tumor
30 cell, while it is exposed to said cytotoxic agent, a
potentiating agent in an amount effective to potentiate
the cytotoxicity of said cytotoxic agent to said cell,

1 wherein said potentiating agent comprises a compound of
formula (1):



or a pharmacologically acceptable salt thereof,

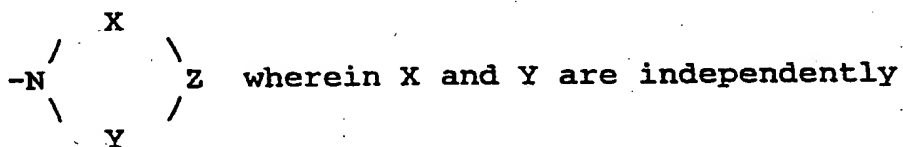
wherein R is -H or $-\text{[C(O)]}_a-\text{(CH}_2\text{)}_b\text{-A}$;

10 wherein a is 0 or 1 and b is an integer from 0 to 6,
provided that a and b are not both zero; and

A is selected from the group consisting of

-NR₁R₂ wherein R₁ and R₂ are independently
alkyl having 1 to 4 carbon atoms, and either or both of

15 R₁ and R₂ are optionally substituted with -OH;



20 alkylene having 1 to 4 carbon atoms, and Z is -O-, -
N(R₃)- or -CH(R₄)-, wherein R₃ is hydrogen or alkyl
having 1 to 4 carbon atoms optionally substituted with a
hydroxyl group, and wherein R₄ is hydrogen or alkyl
having 1 to 4 carbon atoms optionally substituted with a
25 hydroxyl group;

halide; and trihalomethyl.

The present invention further relates to a
composition comprising cytotoxic agent toxic to tumor
cells, and a potentiating agent which potentiates the
30 cytotoxicity of said cytotoxic agent, wherein said
potentiating agent comprises a compound of formula (1)
and wherein said cytotoxic agent and potentiating agent

1 are present in amounts effective to render the
composition cytotoxic to tumor cells.

The present invention still further relates to
a method of killing a tumor cell which comprises
5 administering to said cell a composition as described
above in an amount effective to kill said cell.

As described in more detail below, the present
invention provides novel and effective means for
potentiating the desired cytotoxic effect of anticancer
10 drugs in tumor cells and especially in multidrug-
resistant (MDR) cells.

One preferred group of compounds of the
formula (1) is the N-alkyl derivatives, in which a is 0
in formula (1). Of those compounds wherein a is 0, the
15 more preferred include those in which b is 3 or 4,
denoting unbranched propylene and butylene moieties; R₁
and R₂ each are ethyl, n-propyl, ω-hydroxyethyl, or ω-
hydroxypropyl; X and Y are each -CH₂- or -CH₂CH₂- and,
more preferably, both X and Y are -CH₂CH₂-; and R₃ and
20 R₄ are each -H or ethyl, propyl, e.g. n-propyl, ω-
hydroxyethyl or ω-hydroxypropyl. Other more preferred
embodiments when a is 0 are those derivatives wherein b
is 3 or 4 and A is halogen, preferably chloro.

Another preferred group of compounds of
formula (1) is the N-acyl derivatives, in which a is 1
25 in formula (1). Of those compounds wherein a is 1, the
more preferred include those in which b is 1 or 2, more
preferably 1; R₁ and R₂ are each ethyl, n-propyl, ω-
hydroxyethyl or ω-hydroxypropyl; X and Y are each -CH₂-
or -CH₂CH₂-, and more preferably, both X and Y are -
30 CH₂CH₂-; each of R₃ and R₄ is -H or ethyl, n-propyl, ω-
hydroxyethyl or ω-hydroxypropyl. Other more preferred

embodiments are those in which b is 0 or 1 and A is trihalomethyl, preferably trichloromethyl or trifluoromethyl; and in which b is 1 or 2 and A is halogen, preferably chloro.

As used herein, unless specified otherwise, "alkyl" means saturated, branched or unbranched groups of the formula $-(C_nH_{2n+1})$; "halo" or "halogen" means fluoro, chloro, bromo, and/or iodo; and the optional hydroxyl and halo substituents disclosed herein can be on any carbon of an alkyl or alkylene group.

The compounds of this invention form salts, which are also within the scope of the invention, with various inorganic and organic acids. The pharmacologically acceptable acid addition salts of the compounds of the present invention may be prepared by conventional means, such as by reacting with an appropriate acid providing the desired anion, either in a solvent or medium in which the salt is insoluble, or in water. The salts of strong acids are preferred. As exemplary, but not limiting, of pharmacologically acceptable acid salts are the salts of hydrochloric, hydrobromic, sulfuric, nitric, acetic, fumaric, malic, maleic, tartaric and citric acids.

In general, the synthesis of the N-alkyl and N-acyl derivatives is straightforward. N-alkylation can be achieved in the presence of basic condensing agents like sodium amide. The general procedure for preparing the N-alkyl derivatives of formula (1) consists of the condensation of phenoxazine with the appropriate α , ω -di-alkylhalide in such as $Cl-(CH_2)_b-Br$ wherein b is 1 to 6, in the presence of sodium amide, either in liquid ammonia or in an anhydrous solvent such as toluene or

1 benzene. For instance, the reaction of phenoxazine with
mixed chlorobromoalkanes in the presence of sodium amide
gives reactive N-chloroalkylphenoxazines, which can then
be converted to the desired compound by reaction with
5 an intermediate of the formula $H-(CH_2)_b-A$ wherein b and
A have the meanings set forth above.

More specifically, compounds such as those
described in Examples 1-14 below can be prepared by
first alkylating phenoxazine with 1-bromo-3-
10 chloropropane or 1-bromo-4-chloropropane to produce 10-
(3'-chloropropyl) phenoxazine or 10-(4'-
chlorobutyl)phenoxazine, alkylation being accomplished
by first converting phenoxazine to the anionic species
using the strong base, sodium amide. Iodide-catalyzed
15 nucleophilic substitution of the propyl or butyl
chloride with various secondary amines (e.g. N,N-
diethylamine, N,N-diethanolamine, morpholine,
piperidine, pyrrolidine and 8-hydroxyethyl-piperazine)
by refluxing for about 20 hours with potassium carbonate
in anhydrous acetonitrile affords the free bases of
20 formula (1).

The acyl derivatives of formula (1) can be
synthesized by acylating phenoxazine with a compound of
the formula $Cl-C(O)-C(CH_2)_{o-e}-Cl$ and then reacting the
product with an amine of the formula H-A, wherein A has
25 the meaning given above in anhydrous acetonitrile
containing potassium iodide. The haloacetylphenoxazine
can be prepared by reacting phenoxazine with the
anhydride $(C(halo)_3CO)_2O$.

30 All the compounds described in Examples 1-14
were separated and purified by column chromatography or
recrystallization and dried under high vacuum. The

1 structures were established by UV-, IR, ^1H - and ^{13}C -NMR
and EIMS spectral data, and by elemental analyses. The
physical properties of the compounds are given in Table
I. The UV-spectral data of N-substituted phenoxazines
5 are in close agreement with the spectral characteristics
of analogous heterocycles. The IR bands also indicate
the presence of characteristic functional groups, and
peaks at $1670\text{--}1695\text{ cm}^{-1}$ indicated the presence of $>\text{C}=\text{O}$
group in the acyl derivatives. The ^1H -NMR in CDCl_3 ,
10 typical of phenoxazine compound, showed eight aromatic
protons and the data are in accordance with the
structures assigned. The assignment of protons is fully
supported by the integration curves. The ^{13}C -NMR
spectrum of each N-substituted phenoxazine exhibited
15 size signals representing 12 aromatic carbons. The GC-
Mass spectrum showed an intense molecular ion peak (M^+)
for each of the compounds characteristic of the
phenoxazine type of structure. The spectral data are
consistent with the assigned structures.

20 SYNTHESIS AND ANALYSIS

In the syntheses and experiments described
below, melting points were recorded on a Perkin-Elmer
Model 1320 spectrophotometer, as KBr pellets; UV-spectra
were recorded in MeOH on a Perkin-Elmer Lambda 3B
spectrophotometer. Elemental analyses were performed
25 and found values within 0.4% of theoretical, unless
otherwise noted. Reactions were monitored by tlc. For
tlc, Analtech silica gel GF plates (20 x 20 cm, 250
microns, glass-backed), with petroleum ether-
ethylacetate (9.7:0.3 by volume, system A), and
30 ethylacetate-methanol (9.9:0.1 by volume, system B) as
solvents were used. Column chromatography utilized

1 silica gel Merc grade 60 (230-400 mesh, 60Å). ^1H - and
2 ^{13}C -NMR spectra were recorded in CDCl_3 solution in a 5-
3 mm tube on an IBM NR 200 AF Fourier transform
4 spectrometer with tetramethylsilane as internal
5 standard. Chemical shifts are expressed as δ (ppm)
6 values. The spectrometer was internally locked to the
7 deuterium frequency of the solvent. Electron-impact
8 mass spectra (EIMS) were recorded on a Ribermag R10-10C
9 GC-mass spectrometer with an upper mass limit of 1500
10 AMU. All chemicals and supplies were obtained from
11 standard commercial sources unless otherwise indicated.
12 Phenoxazine, secondary amines indicated in the text, and
13 anhydrous organic solvents were purchased from Aldrich
14 Chemical Co. (Milwaukee, WI). Vincristine sulfate
15 (oncovin) was purchased from Eli Lilly and Co.
16 (Indianapolis, IN), and vinblastine sulfate was from
17 Cetus Corporation (Emeryville, CA). $[\text{G}-^3\text{H}]$ vincristine
18 (sp. act. (specific activity) 7.1 Ci/mmol), and $[\text{G}-^3\text{H}]$ vinblastine
19 (sp. act. 10.1 Ci/mmol) were obtained
20 from Amersham Corporation (Arlington Heights, IL).
21 Verapamil hydrochloride, colchicine, RPMI-1640 medium,
22 powder with glutamine and without sodium bicarbonate
23 were purchased from the Sigma Chemical Co. (St. Louis,
24 MO).

25 The synthesis of representative compounds of
26 formula (1) is described below. Each of the indicated
27 compounds in these Examples is considered a preferred
28 embodiment of the present invention.

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EXAMPLE 1

1 10-(3'-chloropropyl)-phenoxazine. To a
suspension of sodium amide (1.72 g) in 100 ml of liquid
ammonia, 7g (0.038 mol) of phenoxazine was added. After
5 stirring for 30 minutes, 6.3 g (0.04 mol., 3.96 mL) of
1-bromo-3-chloropropane was added slowly with constant
stirring. After one more hour, ammonia was allowed to
evaporate and solid ice pieces were added carefully
followed by cold water. When the reaction ceased, the
10 mixture was extracted three times with ether. The ether
solution was washed three times with water, dried over
anhydrous sodium sulfate and evaporated. The residue
was chromatographed on silica gel. Petroleum ether-
ethylacetate (9 mL + 3 mL) eluted the pure title
15 compound (7.94 g) as white crystals. $VU-\lambda_{max}$ (MeOH)
218, 238 and 321 nm; IR (KBr) 3070, 2860, 1630, 1490,
1380, 1275, 920, 815 and 740 cm^{-1} ; 1H -NMR (δ) 6.47-6.82
(m, 8H, ArH, H_1-H_4 and H_6-H_9), 2.11 (m, 2H, H_1), 3.63
(m, 2H, H_K), and 3.69 (m, 2H, H_m); ^{13}C -NMR (1H
20 decoupled) 111.23 (C_1 and C_9), 115.50 (C_4 and C_6),
121.07 (C_3 and C_7), 123.70 (C_2 and C_8), 133.03 (C_1 and
 C_9), 144.92 (C_4 and C_6), 27.82 (C_1), 41.09 (C_K) and
42.63 (C_m); EIMS (m/z) 259 (M^+).

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EXAMPLE 2

1 10-(3'-diethylaminopropyl)phenoxazine. 1g
 (4.31 mmol) of the product of Example 1 was dissolved in
 150 mL of anhydrous acetonitrile, and 1.5 g KI, 2.13 g
5 K₂CO₃ and 1.6 mL (15.4 mmol) of N,N-diethylamine were
 added. The mixture was refluxed overnight until a
 substantial amount of product was formed (TLC, System B,
 R_f = 0.40). The reaction mixture was diluted with water
 and extracted with ether three times. The ether layer
10 was washed with water and dried over anhydrous Na₂SO₄
 and evaporated. The crude oil was subjected to column
 chromatography for purification. Ethylacetate-petroleum
 ether (50 mL + 50 mL) eluted the title compound as the
 free base as a colorless oil, which was dried and used
15 for NMR studies. An ethereal solution of the free base
 was treated with an excess of tartaric acid to separate
 the hygroscopic tartrate salt (1.2 g). UV-λ_{max} (MeOH)
 215, 238 and 320 nm; IR (CHCl₃) 3378, 2974, 2838, 1453,
 1375, 1155, 973 and 722 cm⁻¹; ¹H-NMR ('δ') 6.51-6.80 (m,
 8H, ArH, H₁-H₄ and H₆-H₉), 1.16 (t, 6H, H_c and H_d), 1.70
20 (m, 2H, H₁), 2.50 (q, 4H, H_a and H_b, J=7 Hz), 3.42-3.63
 (m, 4H, H_x and H_m); ¹³C-NMR 111.54 (C₁ and C₉), 115.49
 (C₄ and C₆), 121.21 (C₃ and C₇), 123.85 (C₂ and C₈),
 132.72 (C₁ and C₉), 144.95 (C₄ and C₆), 8.21 (C_c and
 C_d), 19.90 (C₁), 40.72 (C_a and C_b), 45.87 (C_m), and
25 48.50 (C_x); EIMS (m/z) 296 (M⁺).

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EXAMPLE 3

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10-(3'-bishydroxyethylaminopropyl)phenoxazine.

The procedure used for Example 2 was repeated with 1g, (4.31 mmol) of the product of Example 1, 1.5 g KI, and 1.62 g (15.4 mmol, 1.5 mL) of diethanolamine.

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Recrystallization of the solid in ethylacetate and petroleum ether gave (1.14 g) of the title compound in the pure form. UV- λ_{max} (MeOH) 218, 239, and 322 nm; IR (KBr) 3300, 2960, 2880, 1590, 1490, 1440, 1375, 1270, 1190, 1125, 1075, 1040, 890, 840, and 740 cm^{-1} ; 1H -NMR (' δ ') 6.44-6.78 (m, 8H, ArH, H_1 - H_4 and H_6 - H_9), 1.71-1.82 (m, 2H, H_1), 2.54-2.61 (t, 4H, H_a and H_b , $J = 6$ Hz), 3.39 - 3.68 (m, 8H, H_k , H_c , and H_d and H_m), and 2.95 (s, H_e and H_f , disappearing on D_2O exchange); ^{13}C -NMR 111.37 (C_1 and C_9), 115.33 (C_4 and C_6), 120.80 (C_3 and C_7), 123.66 (C_2 and C_8), 133.25 (C_{10} and C_{11}), 144.99 (C_{12} and C_{13}), 22.42 (C_{14}), 41.83 (C_a and C_b), 52.38 (C_m), 55.91 (C_k) and 59.64 (C_c and C_d); EIMS (m/z) 328 (M^+).

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EXAMPLE 4

1 10-(3'-N-morpholinopropyl)phenoxazine. The
procedure used for Example 2 was repeated with 1g of the
product of Example 1, 1.5 g KI, 2.0 g K₂CO₃ and 1.4 g
5 (15.40 mmol, 1.34 mL) of morpholine. The oily residue
was purified by column chromatography to give the title
compound as a brown oil. An ethereal solution of the
free base was treated with ethereal hydrochloride to
give the hydro-chloride salt (1.07 g). UV- λ_{max} (MeOH)
10 216, 239, and 320 nm; IR (KBr) 3200, 1495, 1380, 1280,
1230, 1135, 1100, 1050, 1020, 980, 870, 830, 760 and 735
cm⁻¹; ¹H-NMR (' δ ') 6.63-6.81 (m, 8H, ArH, H₁-H₄ and H₆-
H₉), 1.78 (m, 2H, H₁), 2.40 (t, 4H, H_a and H_b, J = 12
Hz), 3.45-3.80 (m, 8H, K_x, H_m, H_c and H_d); ¹³C-NMR
15 111.64 (C₁ and C₉), 115.80 (C₄ and C₆), 121.59 (C₃ and
C₇), 123.91 (C₂ and C₈), 133.50 (C₁ and C₉), 145.11
(C₄ and C₆), 20.06 (C₁), 40.93 (C_a and C_b), 51.91
(C_m), 55.20 (C_x), and 63.50 (C_c and C_d); EIMS (m/z) 310
(M⁺).

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EXAMPLE 5

1 10-(3'-N-piperidinopropyl)phenoxazine. The
procedure used for Example 2 was used with 1.12 g (4.31
mmol) of the product of Example 1, 1.5 g IH, 2.4 g K₂CO₃
5 and 1.5 g (17.62 mmol, 1.74 mL) of piperidine. The
product was chromatographed on silica gel with petroleum
ether-ethylacetate (1:1 by volume) to obtain the pure
title compound in the form of an oil. By adding
ethereal hydrochloride to the ether solution of the free
10 base, the hydrochloride salt (1.15 g) was obtained. UV-
 λ_{max} (MeOH) 218, 238 and 320 nm; IR (KBr) 3300, 2940,
2680, 1595, 1495, 1385, 1275, 1160, 1050, 825 and 745
cm⁻¹; ¹H-NMR (' δ ') 6.56-6.86 (m, 8H, ArH, H₁-H₄ and H₆-
H₉), 1.53 (m, 6H, H_c, H_a and H_b), 2.30 (m, 2H, H₁),
2.56-2.67 (m, 4H, H_a and H_b), and 3.45-3.70 (m, 4H, H_k
15 and H_m); ¹³C-NMR 111.65 (C₁ and C₉), 115.62 (C₄ and C₆),
121.38 (C₃ and C₇), 123.88 (C₂ and C₈), 132.73 (C₁ and
C₉), 144.98 (C₄ and C₆), 20.21 (C_e), 21.93 (C_e and
C_d), 22.50 (C₁), 41.05 (C_a and C_b), 53.18 (C_m), and
20 54.62 (C_k); EIMS (m/z) 308 (M⁺).

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EXAMPLE 6

1 10-(3'- β -hydroxyethylpiperazinopropyl)
phenoxazine. The procedure used for Example 2 was
repeated with 1 g (4.31 mmol) of the product of Example
1, 1.5 g KI, 2.12 g K₂CO₃ and 2 g (15.4 mmol, 1.9 mL) of
5 β -hydroxyethylpiperazine. The free base was
recrystallized in petroleum ether-ether mixture (7:3 by
volume) to give 1.16 g of the title compound. UV- λ_{max}
(MeOH) 217, 239 and 322 nm; IR (KBr) 3060, 2820, 1630,
1595, 1495, 1385, 1270, 1160, 1070, 980, 850, 810 and
10 735 cm⁻¹; ¹H-NMR (' δ ') 6.46-6.76 (m, 8H, ArH, H₁-H₄ and
H₆-H₉), 1.74 (m, 2H, H₁), 2.33-2.80 (M, 12H, H_a and H_b,
H_c and H_d, H_e and H_m), 2.79 (s, 1H, H_g, disappearing on
D₂O exchange), 3.47-3.65 (m, 4H, H_k and H_f); ¹³C-NMR
111.34 (C₁ and C₉), 115.24 (C₄ and C₆), 120.66 (C₃ and
15 C₇), 123.50 (C₂ and C₈), 133.30 (C₁ and C₉), 144.83
(C₄ and C₆), 22.58 (C₁), 41.72 (C_m), 52.96 (C_a and
C_b), 53.28 (C_c and C_d), 55.19 (C_k); 57.77 (C_e), and
59.34 (C_f); MS (m/z) 353 (M⁺).

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EXAMPLE 7

1 10-(3'-N-pyrrolidinopropyl)phenoxazine. The
procedure used for Example 2 was repeated with 1g of the
title product of Example 1, 1.5 g KI, 2g K₂CO₃ and 1.1g
5 (15.5 mmol, 1.3 mL) of pyrrolidine. The product was
purified by column chromatography and the oil was
converted into the hydrochloride salt (1.02g). UV- λ_{max}
(MeOH) 217, 239, and 319 nm; IR (KBr) 3300, 2660, 1590,
1490, 1375, 1270, 1130, 920, 820 and 745 cm⁻¹; ¹H-NMR
10 (' δ ') 6.46-6.77 (m, 8H, ArH, H₁-H₄ and H₆-H₉), 2.01-2.17
(t, 4H, H_c and H_d, J = 13 Hz), 2.21 (m, 2H, H₁), 3.06-
3.14 (t, 4H, H_a and H_b), and 3.60-3.67 (m, 4H, H_k and
H_m); ¹³C-NMR 111.60 (C₁ and C₉), 115.66 (C₄ and C₆),
121.40 (C₃ and C₇), 123.85 (C₂ and C₈), 132.73 (C₁ and
15 C₉), 144.98 (C₄ and C₆), 22.25 (C_c and C_d), 23.30
(C₁), 40.90 (C_a and C_b), 52.80 (C_m), and 53.63 (C_k); MS
(m/z) 294 (M⁺).

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EXAMPLE 8

1 10-(4'-chlorobutyl)phenoxazine, (8.4 g) in the
pure form was prepared following the procedure used for
Example 1 with 7g phenoxazine, 1.63 g sodium amide and
5 4.36 mL of 1-bromo-4-chlorobutane (0.038 mol) to produce
the title compound. UV- λ_{max} (MeOH) 200, 212, 238, and
320 nm; IR (KBr) 3060, 2980, 1630, 1590, 1495, 1380,
1280, 1130, 915, 840 and 730 cm^{-1} ; $^1\text{H-NMR}$ (' δ ') 6.36-
6.74 (m, 8H, ArH, $\text{H}_1\text{-H}_4$ and $\text{H}_6\text{-H}_9$), 1.75 (broad, 4H, H_1
and H_m), and 3.38-3.50 (m, 4H, H_x and H_n), $^{13}\text{C-NMR}$
10 111.43 (C_1 and C_9), 115.53 (C_4 and C_6), 121.01 (C_3 and
 C_7), 123.83 (C_2 and C_8), 133.27 (C_1 and C_9), 145.10
(C_4 and C_6), 22.60 (C_m), 29.87 (C_1), 43.27 (C_x), and
44.61 (C_n); EIMS (m/z) 273 (M^+).

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EXAMPLE 9

1 10-(4'-diethylaminobutyl)phenoxazine. The
procedure used for Example 2 was followed with 1g (3.65
mmol) of the product of Example 8, 1.5g KI, 2g K₂CO₃ and
5 1.07 g (14.63 mmol, 1.5 mL) of N,N-diethylamine to
obtain the indicated product. The oily product was
chromato-graphed on the silica gel with CH₃OH-CHCl₃
(3:1) and the hydrochloride salt (.076g) was obtained in
the pure form. UV- λ_{max} (MeOH) 201, 213, 239 and 320 nm;
10 IR (KBr) 3300, 2940, 1590, 1495, 1380, 1270, 1130, 1040,
925 and 750 cm⁻¹; ¹H-NMR (' δ ') 6.47-6.80 (m, 8H, ArH,
H₁-H₄ and H₆-H₉), 1.33 (broad, 6H, H_c and H_d), 1.66-1.91
(m, 4H, H₁ and H_m), 3.05 (very broad, 6H, H_a, H_b and
H_n), and 3.50 (m, 2H, H_k); ¹³C-NMR 111.51 (C₁ and C₉),
115.31 (C₄ and C₆), 120.99 (C₃ and C₇), 123.75 (C₂ and
15 C₈), 132.78 (C₁ and C₉), 144.78 (C₄ and C₆), 8.54
(C_e and C_d), 21.02 (C_m), 22.46 (C₁), 43.05 (C_a and C_b),
46.50 (C_n), and 51.26 (C_k); MS (m/z) 310 (M⁺).

EXAMPLE 10

1 10-(4'-bishydroxyethylaminobutyl) phenoxazine,
as its hydrochloride salt (1.11g) was obtained by
following the procedure of Example 3 with 1g of the
product of Example 8, 1.5g KI and 1.54 g (14.65 mmol,
5 1.4 mL) of N,N-diethanolamine followed by column
chromato-graphy. UV- λ_{max} (MeOH) 204, 210, 238 and 321
nm; IR (KBr) 3280, 2850, 1630, 1590, 1490, 1375, 1270,
1135, 1095, 1065, 1045, 1020, 925, 890, 845, and 740 cm^{-1} ;
10 1H -NMR (' δ ') 6.52-6.84 (m, 8H, ArH, H_1 - H_4 and H_6 - H_9),
1.70-1.98 (m, 4H, H_1 , and H_m), 3.35-3.57 (broad, 10H,
 H_a , H_b , H_n , H_k , H_e and H_f), 3.95 (t, 4H, H_c and H_d ; J =
7 Hz), and 10.3 (H^+); ^{13}C -NMR 110.53 (C_1 and C_9), 114.17
(C_4 and C_6), 119.83 (C_3 and C_7), 122.76 (C_2 and C_8),
131.85 (C_{10} and C_5), 143.60 (C_4 and C_6), 19.98 (C_m),
15 21.10 (C_1), 42.06 (C_n), 52.92 (C_a and C_b), 54.78 (C_k),
and 54.96 (C_e and C_d); EIMS (m/z) 342 (M^+).

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EXAMPLE 11

1 10-(4'-N-morpholinobutyl)phenoxazine. The
procedure used for Example 4 was repeated with 1 g of
the product of Example 8, 1.5g KI, 2g of K₂CO₃ and 1.273
5 g (14.61 mmol, 1.3 mL) of morpholine. The product was
recrystallized in ether-petroleum ether mixture (3:1) to
give the title compound (0.95g). UV- λ_{max} 202, 213,
239, and 321 nm; IR (KBr) 2960, 2810, 1630, 1595, 1495,
1380, 1295, 1220, 1130, 1070, 1010, 970, 920, 870, 855,
10 825, 765 and 745 cm⁻¹; ¹H-NMR (' δ ') 6.53-7.29 (m, 8H,
ArH, H₁-H₄ and H₆-H₉), 1.61-1.74 (m, 4H, H₁ and H_m),
2.40-2.50 (m, 6H, H_a, H_b, and H_n), 3.49 (m, 2H, H_x), and
3.49-3.78 (t, 4H, H_c and H_d, J = 12 Hz); ¹³C-NMR 111.28
(C₁ and C₉), 115.28 (C₄ and C₆), 120.67 (C₃ and C₇),
123.52 (C₂ and C₈), 133.30 (C₁ and C₉), 144.99 (C₄.
15 and C₆), 22.34 (C_m), 23.50 (C₁), 43.63 (C_n), 53.67 (C_a
and C_b), 57.91 (C_x), and 66.97 (C_c and C_d); EIMS (m/z)
324 (M⁺).

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EXAMPLE 12

1 10-(4'-N-piperidinobutyl)phenoxazine. 1g of
the product of Example 8, 1.5g of KI, 2g K₂CO₃ and 1.45g
(17.03 mmol, 1.5 mL) of piperidine were refluxed and
5 processed according to the procedure used for Example
10. Purification by column chromatography afforded the
free amine as a brown oil which was converted into the
hydrochloride salt (1.18 g). UV- λ_{max} 203, 210, 238, and
320 nm; IR (KBr) 3320, 2940, 1625, 1590, 1490, 1380,
1270, 1130, 1060, 955, 840, 820, and 730 cm⁻¹; ¹H-NMR
10 (' δ ') 6.42-6.81 (m, 8H, ArH, H₁-H₄ and H₆-H₉), 1.44-1.82
(m, 6H, H_c, H_d and H_e), 1.98-21.8 (m, H₁ and H_m), 2.70-
2.97 (m, 4H, H_a and H_b), 3.39-3.45 (m, 4H, H_k and H_n)
and 11.54 (H⁺); ¹³C-NMR 111.42 (C₁ and C₉), 115.32 (C₄
and C₆), 120.98 (C₃ and C₇), 123.71 (C₂ and C₈), 132.78
15 (C₁ and C₉), 144.73 (C₄ and C₆), 20.96 (C_e), 21.79
(C_e and C_d), 22.48 (C₁ and C_m), 43.08 (C_a and C_b), 52.91
(C_n), and 56.70 (C_k); EIMS (m/z) 322 (M⁺).

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EXAMPLE 13

1 10-(4'- β -hydroxyethylpiperazinobutyl)
phenoxazine. The procedure used for Example 6 was
repeated with 1 g of the product of Example 8, 1.5g KI,
5 and 1.9g (14.6 mmol, 1.8 mL) of β -
hydroxyethylpiperazine. The oily residue was treated
with 500 μ l of ethylacetate first and then with
petroleum ether (20 mL), when a white crystalline solid
separated out. The solid was recrystallized to give the
10 pure title compound (1.21g). UV- λ_{max} (MeOH) 202, 239,
and 320 nm; IR (KBr) 3060, 2940, 2860, 1590, 1495, 1380,
1225, 1135, 1020, 1005, 935, 880, 830, 780, and 740 cm^{-1} ;
 1H -NMR (δ) 6.46-6.75 (m, 8H, ArH, H_1 - H_4 and H_6 and
 H_9), 1.58 (broad, 4H, H_1 and H_m), 2.36-2.51 (m, 12H, H_a ,
15 H_b , H_c , H_d , H_e and H_n), 3.42 (broad, 3H, H_k , and H_g),
and 3.58-3.63 (t, 2H, H_f , J = 7 Hz); ^{13}C -NMR 111.39 (C_1
and C_9), 115.26 (C_4 and C_6), 120.64 (C_3 and C_7), 123.61
(C_2 and C_8), 133.30 (C_{10} and $C_{10'}$), 144.95 (C_5 and $C_{5'}$),
22.28 (C_{11} and $C_{11'}$), 23.72 (C_{12}), 43.60 (C_a and C_b), 53.11
(C_c and C_d), 57.38 (C_k), 57.96 (C_e) and 59.76 (C_f); EIMS
20 (m/z) 367 (M^+).

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EXAMPLE 14

1 10-(4'-N-pyrrolidinobutyl)phenoxazine. The
experimental steps used for Example 2 were repeated
using 1g of the product of Example 8, 1.5g KI, 2g K₂CO₃
and 1.04g (14.6 mmol, 1.22 mL) of pyrrolidine as
5 reactants. The product was chromatographed on silica
gel with CHCl₃-MeOH (1:1) to give the free amine as a
brown oil. An ether solution of this oil was treated
with ethereal hydrogen chloride to secure the pure
(0.9g) hydrochloride salt. UV- λ_{max} (MeOH) 205, 211, 238
10 and 320 nm; IR (KBr) 3060, 2840, 1590, 1495, 1380, 1295,
1270, 1160, 1090, 1045, 915, 840, 830, 795, and 740 cm⁻¹;
¹H-NMR (' δ ') 6.43-6.79 (m, 8H, ArH, H₁-H₄ and H₆-H₉),
1.64-2.10 (m, 8H, H₁, H_m, H_e and H_d), 2.97-3.17 (m, 6H,
H_a, H_b and H_n), 3.45-3.54 (m, 2H, H_k) and 10.10 (H⁺);
15 ¹³C-NMR 111.43 (C₁ and C₉), 115.41 (C₄ and C₆), 121.01
(C₃ and C₇), 123.73 (C₂ and C₈), 132.89 (C₁ and C₉),
144.87 (C₄ and C₆), 22.47 (C_e and C_d), 23.27 (C₁ and
C_m), 43.14 (C_a and C_b), 53.50 (C_n), and 54.91 (C_k); EIMS
20 (m/z) 308 (M⁺).

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EXAMPLE 15

1 10-(chloroacetyl)phenoxazine. To a solution
of 5g (0.03 mol) of phenoxazine dissolved in 100 mL
anhydrous acetonitrile containing 10 mL of anhydrous
5 ether, was added dropwise 7 mL (9.926 g, 0.088 mol) of
chloroacetyl-chloride with constant stirring. The
reaction mixture was stirred at room temperature for 5H
when white crystalline solid separated out (TLC, system
A, $R_f=0.030$). The crystals were filtered, washed
10 several times with petroleum ether-ether mixture (9:1)
and dried under high vacuum to get 6.03g of the product.
UV- λ_{max} (MeOH) 218, 249, and 287 nm; IR (KBr) 3070,
1675, 1580, 1480, 1410, 1350, 1260, 1210, 1115, 1040,
860, 815, 750 and 660 cm^{-1} ; $^1\text{H-NMR}$ (δ) 7.55-7.61 (m,
15 2H, ArH, H_1 and H_9), 7.12-7.25 (m, 6H, ArH, H_2 - H_4 and
 H_6 - H_8), 4.32 (s, 2H, H_3); $^{13}\text{C-NMR}$ 110.04 (C_1 and C_9),
117.11 (C_4 and C_6), 123.75 (C_3 and C_7), 124.32 (C_2 and
 C_8), 127.60 (C_1 and C_9), 150.95 (C_4 and C_6), 41.51
(C_3), and 170 (C_k); EIMS (m/z) 259 (M^+).

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EXAMPLE 16

1 10-(diethylaminoacetyl)phenoxazine. 1g (3.9
mmol) of the product of Example 15 was dissolved in 150
mL of anhydrous acetonitrile and 1.5g of KI and 1.13 g
5 (15.45 mmol, 1.6 mL) of N,N-diethylamine were added to
it. The reaction mixture was refluxed for 1h when
substantial amount of the product was formed (TLC,
system B, $R_f=0.40$). The mixture was processed as in
Example 2 to get a white crystalline solid which was
10 further recrystallized in ethylacetate and petroleum
ether mixture to get the pure compound (0.86g). UV- λ_{max}
(MeOH) 220, 246, and 287 nm; IR (KBr) 2800, 1685, 1580,
1480, 1320, 1210, 1150, 1060, 1035, 940, 860, 810, 755
and 670 cm^{-1} ; $^1\text{H-NMR}$ (' δ ') 7.53-7.59 (m, 2H, ArH, H_1 and
15 H_9), 7.05-7.20 (m, 6H, ArH, H_2 - H_4 and H_6 - H_8), 0.95 (t,
6H, H_a and H_d , $J=7$ Hz), 2.60 (q, 4H, H_a and H_b), and
3.55 (s, 2H, H_1); $^{13}\text{C-NMR}$ 116.79 (C_1 and C_9), 123.31 (C_4
and C_6), 125.02 (C_3 and C_7), 126.82 (C_2 and C_8), 129.62
(C_1 and C_9), 151.07 (C_4 and C_6), 12.08 (C_a and C_d),
20 47.04 (C_a and C_b), 54.99 (C_1), and 169.84 (C_k); MS (m/z)
296 (M^+).

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EXAMPLE 17

1 10-(N-morpholinoacetyl)phenoxazine. The same
procedure used for Example 16 was employed with 1g of
the product of Example 15, 1.5g KI and 1.347g (16 mmol,
5 1.4 mL) of morpholine. The solid product was
recrystallized in a mixture of ethylacetate, petroleum
ether and ether and the free base was converted into
hydrochloride salt (1.07g) using ethereal hydrochloride.
UV- λ_{max} 213, 246, and 287 nm; IR (KBr) 2980, 2860, 1690,
10 1485, 1440, 1355, 1270, 1180, 1120, 1070, 1005, 900,
870, 855, 760 and 640 cm^{-1} ; $^1\text{H-NMR}$ (' δ ') 7.60 (broad,
2H, ArH, H_1 and H_9), 7.12-7.34 (m, 6H, ArH, H_2 - H_4 and
 H_6 - H_8), 2.40-2.60 (t, 6H, H_a and H_b , $J=12$ Hz), 3.35 (s,
2H, H_1) and 3.50-3.70 (t, 4H, H_c and H_d); $^{13}\text{C-NMR}$ 117.03
15 (C_1 and C_9), 123.90 (C_4 and C_6), 124.98 (C_3 and C_7),
126.95 (C_2 and C_8), 127.91 (C_1 and C_9), 150.54 (C_4
and C_6), 52.41 (C_a and C_b), 57.01 (C_1), 63.23 (C_c and
 C_d), and 163.40 (C_x); MS (m/z) 310 (M^+).

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EXAMPLE 18

1 10-(N-piperidinoacetyl)phenoxazine. The
method employed for Example 17 was used with 1g of the
product of Example 15, 1.5g KI and 1.31g (15.4 mmol,
1.52 mL) of piperidine to get 0.95g of the title
5 compound. UV- λ_{max} (MeOH) 218, 246 and 287 nm; IR (KBr)
2960, 1670, 1610, 1580, 1480, 1370, 1330, 1260, 1190,
1120, 1040, 940, 890, 855, 810, 765, and 655 cm^{-1} ; ^1H -
NMR (' δ ') 7.57-7.61 (m, 2H, ArH, H₁ and H₉), 7.12-7.16
10 (m, 6H, ArH, H₂-H₄ and H₆-H₈), 1.51 (very broad, 6H, H_c,
H_d and H_e), 2.44 (m, 4H, H_a and H_b) and 3.34 (s, 2H,
H₁); ^{13}C -NMR 116.72 (C₁ and C₉), 123.28 (C₄ and C₆),
124.97 (C₃ and C₇), 126.79 (C₂ and C₈), 129.48 (C₁ and
C₉), 151.01 (C₄ and C₆), 23.92 (C_a), 25.93 (C_e and
15 C_d), 54.15 (C_a and C_b), 60.80 (C₁), and 168.92 (C_x);
EIMS (m/z) 308 (M⁺).

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EXAMPLE 19

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10-(8-hydroxyethylpiperazinoacetyl)

phenoxazine. The procedure used for Example 17 was repeated with 1g of the product of Example 15, 1.5g KI and 2g (15.4 mmol, 1.9 mL) of 8-hydroxyethylpiperazine. 5 Recrystallization of the white solid yielded 1.17 g of the title compound. UV_{max} (MeOH) 213, 246 and 287 nm; IR (KBr) 3200, 2940, 1685, 1665, 1480, 1265, 1190, 1160, 945, 855, 765 and 640 cm⁻¹; ¹H-NMR ('δ') 7.53-7.58 (m, 2H, ArH, H₁ and H₉), 7.08-7.25 (m, 6H, ArH, H₂-H₄ and H₆-H₈), 2.48 (m, 1OH, H_a, H_b, H_c, H_d and H_e), 2.70 (s, 1H, H_f, disappearing on D₂O exchange), 3.39 (s, 2H, H₁) and 3.60 (t, 2H, H_f, J=7 Hz); ¹³C-NMR 116.85 (C₁ and C₉), 123.34 (C₄ and C₆), 124.86 (C₃ and C₇), 126.99 (C₂ and C₈), 129.25 (C₁ and C₉), 151.04 (C₄ and C₆), 15 52.70 (C_a and C_b), 52.90 (C_c and C_d), 57.70 (C_f), 59.23 (C₁), 59.80 (C_f), and 168.43 (C_x); EIMS (m/z) 353 (M⁺).

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EXAMPLE 20

1 10-(N-pyrrolidinoacetyl)phenoxazine. The
experimental procedure used for Example 17 was employed
with 1g of the product of Example 15, 1.5g KI and 1.095g
5 (15.4 mmol, 1.3 mL) of pyrrolidine. Purification by
recrystallization afforded 1.02 g of the title compound.
UV- λ_{max} (MeOH) 214, 240, and 286 nm; IR (KBr) 2980,
2820, 1695, 1670, 1480, 1455, 1340, 1270, 1180, 1100,
1040, 985, 905, 855, 755 and 640 cm^{-1} ; $^1\text{H-NMR}$ (' δ ')
10 7.58-7.63 (m, 2H, ArH, H_1 and H_9), 7.07-7.18 (m, 6H,
ArH, H_2 - H_4 and H_6 - H_8), 1.77 (t, 4H, H_c and H_d , $J=7$ Hz),
2.64 (t, 4H, H_a and H_b) and 3.51 (s, 2H, H_1); $^{13}\text{C-NMR}$
116.80 (C_1 and C_9), 123.33 (C_4 and C_6), 125.06 (C_3 and
 C_7), 126.85 (C_2 and C_8), 129.28 (C_1 and C_9), 151.00
15 (C_4 and C_6), 23.73 (C_c and C_d), 53.83 (C_a and C_b),
57.24 (C_1), and 168.92 (C_k); EIMS (m/z) 294 (M^+).

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EXAMPLE 21

1 10-(trifluoroacetyl)phenoxazine. To a
solution of 200 mg of phenoxazine in 10 mL anhydrous
chloroform and 4 mL anhydrous ether, was added 50 μ l of
5 (0.7435g, 3.54 mmol) trifluoroacetic anhydride. The
resulting mixture was stirred at room temperature for 8
hours. The formation of the product was monitored by
TLC (system A). The product solution was then extracted
with chloroform and evaporated. The residue was
10 subjected to column chromatography which afforded the
pure title compound. UV- λ_{max} (MeOH) 212, 238, and 252
nm; IR (KBr) 3375, 1695, 1580, 1480, 1455, 1390, 1290,
1170, 1110, 1030, 965, 890, 850, 800, 760, 730, and 670
cm⁻¹; ¹H-NMR (' δ ') 7.57-7.61 (m, 2H, ArH, H₁ and H₉),
15 7.14-7.32 (m, 6H, ArH, H₂-H₄ and H₆-H₈); ¹³C-NMR 117.20
(C₁ and C₉), 123.83 (C₄ and C₆), 124.34 (C₃ and C₇),
128.34 (C₂ and C₈), 151.04 (C₁ and C₉, and C₄ and
C₆), and >200 ppm (C_x and C₁); EIMS (m/z) 279 (M⁺).

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TABLE I

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PHYSICAL PROPERTIES OF N-(ALKYLAMINO) OR N-ACYLAMINO DERIVATIVES OF PHENOXAZINE		
Product Of Example No.	Yield, %	mp, °C
1	80	53
2	70	ND
3	90	83-84
4	80	198*
5	70	202*
6	85	108
7	75	158-159*
8	80	46
9	60	127*
10	80	115*
11	80	89 187*
12	90	190*
13	90	114
14	70	170*
15	85	143-144
16	75	39
17	80	130*
18	80	110-111
19	85	70-71
20	80	96-98*
21	70	90
* - HCl salt		

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1 The potentiating agent is preferably
administered by infusion in solution in sterile water.
The potentiating agents as hydrochloride salts can be
dissolved in sterile water. The agents as bases can be
5 solubilized in 1N hydrochloric acid, following which the
solution is back titrated with sodium hydroxide to
provide a final pH between 7 and 8.

Cytotoxic agents whose cytotoxicity would be
potentiated by agents within the scope of this invention
10 include VCR, VLB, doxorubicin, colchicine, actinomycin
D, daunomycin, M-AMSA, and other anthracyclic compounds.

The potentiating agent is administered to
tumor cells which are exposed to one or more cytotoxic
agents. By "exposed" is meant that the cytotoxic agent
has been administered simultaneously with the
15 potentiating agent, and/or is administered subsequently
to the administration of the potentiating agent, so long
as at least some of the cytotoxic agent(s) is present in
the tumor cell when the potentiating agent is present in
the tumor cell. The cytotoxic agent should not be
20 administered before the potentiating agent. Preferably,
the cytotoxic agent is administered when the
potentiating agent concentration reaches steady state
during administration by infusion.

It will be recognized that the amount of
25 potentiating agent to be administered will vary between
hosts, between cytotoxic agents and between potentiating
agents, but the effective amounts can readily be
ascertained by those of ordinary skill in this field.
As guidance one can refer to the data in Examples 22-24
30 as well as the following Table. In general, though,
effective amounts to potentiate cytotoxic agents are

1 about 2000-3000 moles of potentiating agent per mole of
VCR; about 1,000-2,000 moles of potentiating agent per
mole of VLB; and about 25-35 moles of potentiating agent
per mole of VP-16 (Etoposide). These values, and the
5 corresponding values for any other cytotoxic agents, can
readily be converted if desired into dosages per host
body weight by calculation based on the dosages for the
cytotoxic agent of interest. The in vitro techniques
described herein can be employed to determine the
10 effectiveness of any particular potentiating agent with
any given cytotoxic agent or agents.

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EXAMPLE 22

1 Table II below gives representative in vivo
values of the molar ratios (shown below as "compound:
(cytotoxic agent)") of potentiating agent to cytotoxic
5 agent for compounds within the scope of this invention.
Vincristine (VCR) was administered to mice at 3 mg/kg
(3.25 μ mol/kg); vinblastine (VLB) was at 5 mg/kg (5.5
 μ mol/kg); VP-16 (Etoposide) was at 50 mg/kg/day for 3
days (0.255 mmol/kg total). The compound number is the
10 number of the example in which the potentiating agent
was prepared.

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TABLE II

Compound No.	Compound:VCR	Compound:VLB	Compound:VP-16
3	2345	1388	29.9
4	2483	1469	31.6
11	2375	1405	30.3
18	2498	1478	31.8

EXAMPLE 23Evaluation of N-substituted
Phenoxazines For Anti-MDR activity

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5 A cloned line of human colon adenocarcinoma, GC₃/Cl³¹, which is intrinsically resistant to VCR (\approx 4-fold relative to KB-3-1), was routinely grown at 37°C in antibiotic-free RPMI-1640 medium supplemented with 2 mM glutamine and 10% FBS (Hyclone Laboratories, Inc., Logan, UT) in a humidified atmosphere of 5% CO₂ and 95% air. Human epidermoid carcinoma KB-3-1 cells and a
10 colchicine selected MDR variant, KBCh^R-8-5, were obtained which was cross-resistant to VCR (45-fold) and VLB (6.3-fold); it was grown in monolayer culture at 37°C in DMEM with 10% FBS and L-glutamine in a
15 humidified atmosphere of 10% CO₂ in air. The resistance of the KBCh^R-8-5 cells was maintained by culturing them with colchicine (10 ng/ml).

Then, 2 mL of cell suspensions (2×10^6) were plated in 35 x 10 mm style "easy grip" culture dishes (Becton Dickinson Co., Lincoln Park, NJ). Cells were
20 allowed to attach to plastic overnight at 37°C. Medium was aspirated and cells were washed with (2 x 2 mL) physiologic tris (PT) buffer. Monolayers were incubated at room temperature for 10 minutes in PT buffer prior to aspiration and adding 1 mL of serum-free RPMI-1640 Hepes
25 buffer (10.4g RPMI-1640 medium in 1L of 25 mM Hepes, pH 7.4) containing 70.4 nM [³H] VCR (sp.act. 7.1 ci/mmol) or 49.5 nM [³H] VLB (sp. act. 10.1 Ci/mmol) with or without a compound of Examples 1-21 (100 μ M) or VRP dissolved in H₂O dissolved in DMSO (final culture
30 concentration <0.1% DMSO). After 2h of incubation at room temperature, medium was rapidly aspirated to

1 terminate drug accumulation, and monolayers were washed
four times with ice-cold PBS (g/L: NaCl 8.0;
Na₂HPO₄.12H₂O, 2.9; KCl 0.2; KH₂PO₄, 0.2) and drained.
To each dish, 1 ml of trypsin-EDTA (0.05% trypsin, 0.53
5 mM EDTA) was added. After 1 minute, monolayers were
triturated to give a uniform suspension of cells, and
radioactivity in 0.75 ml was determined by scintillation
counting. Cell number per dish was determined on 200 µl
of suspension using the method of Butler, and amounts of
intracellular VCR or VLB were determined. The results
10 are set forth in Table III, in which the compound number
is the number of the Example in which the compound (or
"modulator" or "potentiating agent") was prepared.

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TABLE III

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EFFECTS OF N-SUBSTITUTED PHENOXAZINES ON MDR ACTIVITY				
Vinca Accumulation ^a (% control)				
KB Ch ^R -8-5 Cells			GC ₃ /Cl Cells	
Modulator Compound Number	VCR	VLB	VCR	VLB
1	454	342	846	570
2	546	2123	439	1025
3	473	1666	464	1070
4	742	1717	634	960
5	435	1227	282	633
6	343	824	368	879
7	408	969	250	757
8	398	792	317	361
9	211	697	325	737
10	92	403	382	1165
11	702	2684	477	1175
12	196	1071	416	1121
13	91	188	543	1340
14	198	477	412	1315
15	138	236	171	284
16	184	953	160	305
17	290	674	213	298
18	326	2023	177	446
19	280	776	157	426
20	188	776	151	296
21	415	827	230	222
Verapamil	402	1124	178	238
^a $\frac{\text{vinca uptake with modulator}}{\text{vinca uptake without modulator}} \times 100$				
^b Compounds were tested at 100 μ M. All values represent the mean of two separate experiments with a SD of less than 10% of the mean; each experiment was done in triplicate.				

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EXAMPLE 24

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Evaluation of N-substituted Phenoxazines
Cytotoxicity To Tumor Cells

5 The KBCh^R-8-5 cells were plated in triplicate
at a density of 1000 cells per well and GC₃ at 3000
cells per well in Falcon 6-well flat-bottom tissue
culture plates (Becton Dickinson Co., Lincoln Park, NJ).
After 24h, incubation medium was replaced with 3 mL of
fresh medium containing compounds 1-4 or 10-14 or 18 at
10 concentrations ranging from 1-100 μ m (final culture
concentration, 0.1% DMSO), and cells were incubated at
37°C for a further 7 days. The medium was aspirated and
cells were washed once with 2 mL of 0.9% saline and
dried overnight. Colonies were stained with 1 mL of
15 0.1% crystal violet followed by washing twice with
distilled water and were counted using an automated
ARTEK Model 880 colony counter. The IC₅₀ values were
determined from concentration-percent-cell-survival
curves and were defined as the concentrations of
phenoxazines required for 50% reduction in colonies
20 compared to controls. The results of these measurements
are set forth in Table IV.

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TABLE IV

CYTOTOXICITY OF N-SUBSTITUTED PHENOXAZINES		
IC ₅₀ , ^a μ M		
Compound Number	KBCh ^R -8-5	GC ₃ /CL
1	57	83.00
2	15	ND
3	38	37
4	73	40
10	<10	16
11	18	27
12	<10	7
13	<10	7
14	<10	8
18	73	ND
^a IC ₅₀ is the concentration required to produce 50% reduction in clonogenic survival of GC ₃ /CL and KBCh ^R -8-5 cells under the conditions described in Example 23.		

EXAMPLE 25

1

Effect Of N-substituted Phenoxazines
On In Vitro Cytotoxicity Of VLB And VCR

Tumor cells were treated with graded
5 concentrations of VCR and VLB in the absence or presence
of nontoxic concentrations of the products of Examples
1, 3, 4 and 18. The plates were then transferred to a
CO₂ incubator and, after further incubation for 7 days
at 37°C, colonies were enumerated as described in
10 Example 23. The results are set forth in Table V.

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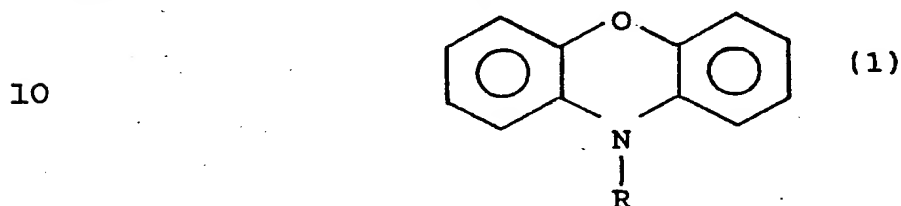
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TABLE V

Potentiation Of Cytotoxicity Of Vincristine And Vinblastine By N-substituted Phenoxazines Against GC ₃ /Cl And KBCh ^a -8-5 Cells					
IC ₅₀ Values, nM					
KB Ch ^a -8-5 Cells			GC ₃ /Cl Cells		
Compound Number	Concentration of Modulator ^a (μ M)	VCR	VLB	VCR	VLB
no modulator	-	32.0	20.0	27.0	7.4
1	50	-	-	9.0	-
3	25	-	2.7	-	2.0
4	25	1.2	1.6	0.85	2.0
18	49	-	2.3	-	2.2
^a IC ₅₀ concentration of modulator					

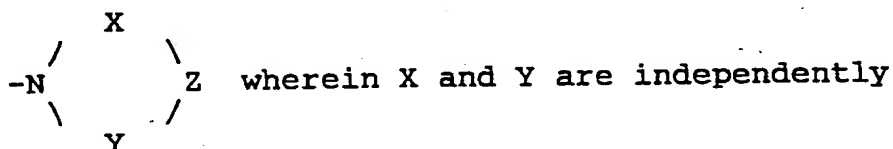
1 WHAT IS CLAIMED IS:

1. A method of potentiating the cytotoxicity of an agent cytotoxic to a tumor cell, comprising administering to said tumor cell, while it is exposed to said cytotoxic agent, a potentiating agent in an amount effective to potentiate the cytotoxicity of said cytotoxic agent to said cell, wherein said potentiating agent comprises a compound of the formula (1):



or a pharmacologically acceptable salt thereof,
 15 wherein R is -H or $-[C(O)]_a-(CH_2)_b-A$; wherein a is 0 or 1 and b is an integer from 0 to 6, provided that a and b are not both zero; and

A is selected from the group consisting of
 $-NR_1R_2$ wherein R_1 and R_2 are independently
 20 alkyl having 1 to 4 carbon atoms, and either or both of R_1 and R_2 are optionally substituted with -OH;



25 alkylene having 1 to 4 carbon atoms, and Z is -O-,
 $-N(R_3)-$ or $-CH(R_4)-$, wherein R_3 is hydrogen or alkyl having 1 to 4 carbon atoms optionally substituted with a hydroxyl group, and wherein R_4 is hydrogen or alkyl
 30 having 1 to 4 carbon atoms optionally substituted with a hydroxyl group;
 halide; and trihalomethyl.

1 2. The method of Claim 1 wherein said tumor cell is present in a living host.

 3. The method of Claim 1 wherein said cytotoxic agent is selected from the group consisting of
5 vincristine, vinblastine, etoposide, doxorubicin, colchicine, actinomycin D, daunomycin, m-AMSA, and mixtures thereof.

 4. The method of Claim 1 wherein said tumor cell exhibits multiple drug resistance.

10 5. The method of Claim 1 wherein a is zero; b is 3 or 4; R₁ and R₂ are independently selected from the group consisting of ethyl, propyl, ω-hydroxyethyl, and ω-hydroxypropyl; X and Y are each independently selected from the group consisting of -CH₂- and -CH₂CH₂-; and R₃
15 and R₄ are independently selected from the group consisting of -H, ethyl, propyl, ω-hydroxyethyl, and ω-hydroxypropyl.

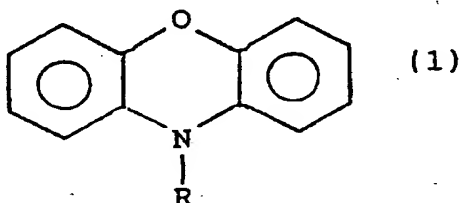
 6. The method of Claim 5 wherein said potentiating agent is 10-(3'-chloropropyl)-phenoxazine, 10-(3'-diethylaminopropyl)-phenoxazine, 10-(3'-
20 bishydroxyethylaminopropyl)-phenoxazine, 10-(3'-N-morpholinopropyl)-phenoxazine, 10-(3'-N-piperidinopropyl)-phenoxazine, 10-(3'-β-hydroxyethylpiperazinopropyl)-phenoxazine, (10-(3'-N-pyrrolidinopropyl)-phenoxazine, 10-(4'-chlorobutyl)-
25 phenoxazine, 10-(4'-diethylaminobutyl)-phenoxazine, 10-(4'-bishydroxyethylaminobutyl)-phenoxazine, 10-(4'-N-morpholinobutyl)-phenoxazine, 10-(4'-piperidinobutyl)-phenoxazine, 10-(4'-β-hydroxyethylpiperazinobutyl)-phenoxazine, 10-(4'-N-pyrrolidinobutyl)-phenoxazine or
30 pharmacologically acceptable salts thereof.

 7. The method of Claim 1 wherein a is 1.

1 8. The method of Claim 7 wherein b is 1 or 2;
2 R₁ and R₂ are independently selected from the group
3 consisting of ethyl, propyl, ω-hydroxyethyl, and ω-
4 hydroxypropyl; X and Y are each independently selected
5 from the group consisting of -CH₂- and -CH₂CH₂-; and R₃
6 and R₄ are independently selected from the group
7 consisting of -H, ethyl, propyl, ω-hydroxyethyl, and ω-
8 hydroxypropyl.

9 9. The method of Claim 8 wherein said
10 potentiating agent is 10-(chloroacetyl)-phenoxazine, 10-
11 (diethylaminoacetyl)-phenoxazine, 10-(N-
12 morpholinoacetyl)-phenoxazine, 10-(N-piperidinoacetyl)-
13 phenoxazine, 10-(8-hydroxyethylpiperazinoacetyl)-
14 phenoxazine, 10-(N-pyrrolidinoacetyl)-phenoxazine, 10-
15 (trifluoroacetyl)-phenoxazine or pharmacologically
16 acceptable salts thereof.

17 10. A composition comprising a cytotoxic
18 agent toxic to tumor cells, and a potentiating agent
19 which potentiates the cytotoxicity of said cytotoxic
20 agent, wherein said potentiating agent comprises a
21 compound of the formula (1)



26 or a pharmacologically acceptable salt thereof,
27 wherein R is -H or -[C(O)]_a-(CH₂)_b-A;
28 wherein a is 0 or 1 and b is an integer from 0 to 6,
29 provided that a and b are not both zero; and
30 A is selected from the group consisting of

1 $\text{-NR}_1\text{R}_2$ wherein R_1 and R_2 are independently
alkyl having 1 to 4 carbon atoms, and either or both of
 R_1 and R_2 are optionally substituted with -OH ;

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$$\begin{array}{c} \text{X} \\ / \quad \backslash \\ \text{-N} \quad \text{Z} \\ \backslash \quad / \\ \text{Y} \end{array}$$
 wherein X and Y are independently

alkylene having 1 to 4 carbon atoms, and Z is -O- , $\text{-N(R}_3\text{)-}$ or $\text{-CH(R}_4\text{)-}$, wherein R_3 is hydrogen or alkyl
10 having 1 to 4 carbon atoms optionally substituted with a
hydroxyl group, and wherein R_4 is hydrogen or alkyl
having 1 to 4 carbon atoms optionally substituted with a
hydroxyl group;

halide; and trihalomethyl;

15 wherein said cytotoxic agent and potentiating
agent are present in amounts effective to render the
composition cytotoxic to tumor cells.

11. The composition of Claim 10 wherein said
cytotoxic agent is selected from the group consisting of
vincristine, vinblastine, etoposide, doxorubicin,
20 colchicine, actinomycin D, daunomycin, m-AMSA, and
mixtures thereof.

12. The composition of Claim 10 wherein a is
zero; b is 3 or 4; R_1 and R_2 are independently selected
from the group consisting of ethyl, propyl, ω -
25 hydroxyethyl, and ω -hydroxypropyl; X and Y are each
independently selected from the group consisting of $\text{-CH}_2\text{-}$ and $\text{-CH}_2\text{CH}_2\text{-}$; and R_3 and R_4 are independently
selected from the group consisting of -H , ethyl, propyl,
 ω -hydroxyethyl, and ω -hydroxypropyl.

30 13. The composition of Claim 12 wherein said
potentiating agent is 10-(3'-chloropropyl)-phenoxazine,

1 10-(3'-diethylaminopropyl)-phenoxazine, 10-(3'-
bishydroxyethylaminopropyl)-phenoxazine, 10-(3'-N-
morpholinopropyl)-phenoxazine, 10-(3'-N-
piperidinopropyl)-phenoxazine, 10-(3'-β-
5 hydroxyethylpiperazinopropyl)-phenoxazine, (10-(3'-N-
pyrrolidinopropyl)-phenoxazine, 10-(4'-chlorobutyl)-
phenoxazine, 10-(4'-diethylaminobutyl)-phenoxazine, 10-
(4'-bishydroxyethylaminobutyl)-phenoxazine, 10-(4'-N-
morpholinobutyl)-phenoxazine, 10-(4'-piperidinobutyl)-
10 phenoxazine, 10-(4'-β-hydroxyethylpiperazinobutyl)-
phenoxazine, 10-(4'-N-pyrrolidinobutyl)-phenoxazine or
pharmacologically acceptable salts thereof.

14. The composition of Claim 10 wherein a is
1; b is 1 or 2; R₁ and R₂ are independently selected
from the group consisting of ethyl, propyl, ω-
15 hydroxyethyl, and ω-hydroxypropyl; wherein X and Y are
each independently selected from the group consisting of
-CH₂- and -CH₂CH₂-; and R₃ and R₄ are independently
selected from the group consisting of -H, ethyl, propyl,
ω-hydroxyethyl, and ω-hydroxypropyl.
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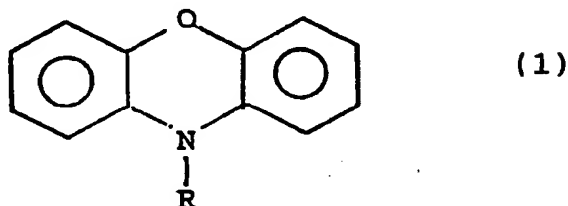
15. The composition of Claim 14 wherein said
potentiating agent is 10-(chloroacetyl)-phenoxazine, 10-
(diethylaminoacetyl)-phenoxazine, 10-(N-
morpholinoacetyl)-phenoxazine, 10-(N-piperidinoacetyl)-
phenoxazine, 10-(β-hydroxyethylpiperazinoacetyl)-
25 phenoxazine, 10-(N-pyrrolidinoacetyl)-phenoxazine, 10-
(trifluoroacetyl)-phenoxazine or pharmacologically
acceptable salts thereof.

16. A method of killing a tumor cell which
comprises administering to said cell a composition
30 according to Claim 10 in an amount effective to kill
said cell.

17. The method of Claim 16 wherein said tumor cell is present in a living host.

18. The method of Claim 16 wherein said tumor cell exhibits multiple drug resistance.

19. A compound of the formula (1)



and pharmacologically acceptable salts thereof, wherein R is $-[C(O)]_a-(CH_2)_b-A$; wherein a is 0 or 1 and b is an integer from 0 to 6, provided that a and b are not both zero; and

A is selected from the group consisting of $-NR_1R_2$ wherein R_1 and R_2 are independently alkyl having 1 to 4 carbon atoms, and either or both of R_1 and R_2 are optionally substituted with $-OH$;

20
$$\begin{array}{c} \text{X} \\ / \quad \backslash \\ -N \quad \quad Z \\ \backslash \quad / \\ \text{Y} \end{array}$$
 wherein X and Y are independently

alkylene having 1 to 4 carbon atoms, and Z is $-O-$, $-N(R_3)-$ or $-CH(R_4)-$, wherein R_3 is hydrogen or alkyl having 1 to 4 carbon atoms optionally substituted with a hydroxyl group, and wherein R_4 is hydrogen or alkyl having 1 to 4 carbon atoms optionally substituted with a hydroxyl group;

halide; and trihalomethyl.

20. A compound or salt according to Claim 19 wherein a is zero; b is 3 or 4; R_1 and R_2 are

1 independently selected from the group consisting of
ethyl, propyl, ω -hydroxyethyl, and ω -hydroxypropyl; X
and Y are each independently selected from the group
consisting of $-\text{CH}_2-$ and $-\text{CH}_2\text{CH}_2-$; and R_3 and R_4 are
5 independently selected from the group consisting of $-\text{H}$,
ethyl, propyl, ω -hydroxyethyl, and ω -hydroxypropyl.

21. The compound according to Claim 20 which
is 10-(3'-chloropropyl)-phenoxazine, 10-(3'-
diethylaminopropyl)-phenoxazine, 10-(3'-
10 bishydroxyethylaminopropyl)-phenoxazine, 10-(3'-N-
morpholinopropyl)-phenoxazine, 10-(3'-N-
piperidinopropyl)-phenoxazine, 10-(3'-8-
hydroxyethylpiperazinopropyl)-phenoxazine, (10-(3'-N-
pyrrolidinopropyl)-phenoxazine, 10-(4'-chlorobutyl)-
phenoxazine, 10-(4'-diethylaminobutyl)-phenoxazine, 10-
15 (4'-bishydroxyethylaminobutyl)-phenoxazine, 10-(4'-N-
morpholinobutyl)-phenoxazine, 10-(4'-piperidinobutyl)-
phenoxazine 10-(4'-8-hydroxyethylpiperazinobutyl)-
phenoxazine, 10-(4'-N-pyrrolidinobutyl)-phenoxazine or
pharmacologically acceptable salts thereof.

20 22. A compound or salt according to Claim 19
wherein a is 1; b is 1 or 2; R_1 and R_2 are independently
selected from the group consisting of ethyl, propyl, ω -
hydroxyethyl, and ω -hydroxypropyl; X and Y are each
independently selected from the group consisting of -
25 CH_2- and $-\text{CH}_2\text{CH}_2-$; and R_3 and R_4 are independently
selected from the group consisting of $-\text{H}$, ethyl, propyl,
 ω -hydroxyethyl, and ω -hydroxypropyl.

23. The compound according to Claim 22 which
is 10-(chloroacetyl)-phenoxazine, 10-
30 (diethylaminoacetyl)-phenoxazine, 10-(N-
morpholinoacetyl)-phenoxazine, 10-(N-piperidinoacetyl)-

1 phenoxazine, 10-(8-hydroxyethylpiperazinoacetyl)-
phenoxazine, 10-(N-pyrrolidinoacetyl)-phenoxazine, 10-
(trifluoroacetyl)-phenoxazine or pharmacologically
acceptable salts thereof.

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 92/06681

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)⁶

According to International Patent Classification (IPC) or to both National Classification and IPC
 Int.Cl.5 A 61 K 31/535 C 07 D 265/38

II. FIELDS SEARCHED

Minimum Documentation Searched⁷

Classification System	Classification Symbols
Int.Cl.5	A 61 K

Documentation Searched other than Minimum Documentation
to the extent that such Documents are Included in the Fields Searched⁸

III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹

Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	Cancer Communications, vol. 2; no. 7, 1990, Pergamon Press, (US), K.N. THIMMAIAH et al.: "Structural determinants of phenoxazine type compounds required to modulate the accumulation of vinblastine and vincristine in multidrug-resistant cell lines", pages 249-259, see abstract; page 249; page 251, table 1; pages 257-258	1-4, 10, 11, 16- 18
Y	<div style="text-align: center;">---</div> <div style="text-align: center;">---</div> <div style="text-align: center;">-/-</div>	5-9

¹⁰ Special categories of cited documents: ¹⁰

"A" document defining the general state of the art which is not
considered to be of particular relevance

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filing date

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which is cited to establish the publication date of another
citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or
other means

"P" document published prior to the international filing date but
later than the priority date claimed

"T" later document published after the international filing date
or priority date and not in conflict with the application but
cited to understand the principle or theory underlying the
invention

"X" document of particular relevance; the claimed invention
cannot be considered novel or cannot be considered to
involve an inventive step

"Y" document of particular relevance; the claimed invention
cannot be considered to involve an inventive step when the
document is combined with one or more other such docu-
ments, such combination being obvious to a person skilled
in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

16-11-1992

Date of Mailing of this International Search Report

07. 12. 92

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

Dagmar Frank

Mme Dagmar FRANK

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		Relevant to Claim No.
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	
X	Gann, The Japanese Journal of Cancer Research, vol. 64, no. 4, August 1973, F. KANZAWA et al.: "Antitumor activity of haloacetylcarbazole derivatives", pages 391-396, see pages 392-393, tables I-II; page 394 ---	1-2,7,9 ,19,23
X	GB,A, 850334 (CHAS. PFIZER & CO. INC.) 5 October 1960, see pages 1-3; claims 10-12,14 ---	19-21
Y	---	5-9
X	BE,A, 569697 (S.A. RECHERCHE ET INDUSTRIE THERAPEUTIQUES) 24 January 1959, see pages 4,8; claims 9,10,18,22,23 ---	19-21
Y	---	5-9
T	Journal of Medicinal Chemistry, vol. 35, no. 18, 4 September 1992, American Chemical Society, K.N. THIMMAIAH et al.: "Synthesis and chemical characterization of N-substituted phenoxazines directed toward reversing vinca alkaloid resistance in multidrug-resistant cancer cells", pages 3358-3364, see whole article -----	1-23

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9206681

SA 63482

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 27/11/92. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A- 850334		None	
BE-A- 569697		None	